Issued May 11, 1907.

U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN No. 99.

A. D. MELVIN, CHIEF OF BUREAU.

# THE DANGER FROM TUBERCLE BACILLI IN THE ENVIRONMENT OF TUBERCULOUS CATTLE.



BY

E. C. SCHROEDER, M. D. V.,
*Superintendent of Experiment Station,*

AND

W. E. COTTON,
*Expert Assistant at Experiment Station*



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GOVERNMENT PRINTING OFFICE.
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# LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
*Washington, D. C., April 4, 1907.*

Sir: I have the honor to transmit herewith, and to recommend for publication as Bulletin No. 99 of this Bureau, an article entitled "The Danger from Tubercle Bacilli in the Environment of Tuberculous Cattle," by Dr. E. C. Schroeder and W. E. Cotton, of the Bureau Experiment Station.

The investigations with which the article deals show that tuberculous cattle, before they lose their general appearance of health, often pass feces heavily infected with tubercle bacilli, and that the same is true of healthy cattle that are permitted to swallow tubercle bacilli in their feed or in their drinking water. When test animals are inoculated with such feces, or with milk soiled with such feces, they commonly become affected with tuberculosis.

The work as a whole shows that the general condition or appearance of a tuberculous animal gives no indication as to the time when it will begin to distribute tubercle bacilli and become dangerous; that the milk from all tuberculous cattle, irrespective of the condition of their udders, should be regarded as dangerous, and that even the milk of healthy cows if it is drawn in the environment of tuberculous cattle may contain tubercle bacilli.

Since the tuberculin test is the only practical means of detecting tuberculosis in the live animal before an advanced stage is reached, the importance of testing dairy cows with tuberculin and removing every reacting animal is again emphasized.

Respectfully,

A. D. Melvin,
*Chief of Bureau.*

Hon. James Wilson,
*Secretary of Agriculture.*

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# THE DANGER FROM TUBERCLE BACILLI IN THE ENVIRONMENT OF TUBERCULOUS CATTLE.

## INTRODUCTION.

The manner in which tubercle bacilli are thrown out by and reach the environment of persons affected with tuberculosis has been carefully studied; consequently, we know with practical certainty that all cases of human tuberculosis may be divided into two classes—"open," or dangerous, and "closed," or less dangerous. A "closed" may at any time become an "open" tuberculosis, but it is from the latter only, which includes all persons whose lungs, air passages and immediately related structures are affected, that tubercule bacilli are disseminated in a way dangerous to health. This is the view sanctioned by Dr. Robert Koch in his "Nobel lecture," delivered at Stockholm on December 10, 1905.[a]

Concerning tuberculous animals our knowledge is not so definite, but it is of the highest importance that we should know how tubercle bacilli are scattered by them, too, not that we may establish an "open" and a "closed" class, but to enable us to know how we can best prevent the transmission of tuberculosis from animal to animal and from animal to man.

Many prominent investigators are convinced that tuberculosis of animals constitutes a serious danger for man; others are not; but all agree that it is an occasional danger, more frequently for children, whose diet consists largely of milk, than for adults. While this matter awaits a solution and there is not perfect harmony among authorities, we may reasonably assume that it is better to guard against exposure to infectious material scattered by persons and animals than only against that scattered by persons. Protective measures that take into account the clearly defined menace and neglect the highly probable danger are insufficient, especially when the probable danger is, as in this case, believed to be actual by many men who are competent to judge.

But even if persons are eventually shown to be comparatively safe against infection from tuberculous animals, the manner of dissemination is nevertheless an important economic problem, because animals have been repeatedly shown to be more susceptible to tuberculous in-

[a] Translation in the Lancet of May 26, 1906.

fection from animals than from man, and tuberculosis among animals is, from the stock owner's point of view, one of the very serious conditions with which the animal industry has to deal.

In this article are recorded some observations on the tubercle bacilli expelled by cattle, and an attempt is made to show how these bacilli may be scattered and become a great danger to animals, and, as we believe, also to man.

## DESCRIPTION OF CATTLE USED IN EXPERIMENTS.

The cattle from which the material was obtained for the observations require the following short description:

Cow No. 1 has been affected with tuberculosis for several years. Aside from a tuberculin test, which showed her to be tuberculous, there is nothing in her condition that would condemn her for use as a dairy cow.

Cow No. 113 is affected with advanced tuberculosis. She is still alive, quite thin, and has a severe and frequent cough.

Cows Nos. 84, 374, and 384 are three healthy animals that were fed a small amount of culture of tubercle bacilli daily in their drinking water during the time the observations were in progress.

Cow No. 325 and steer No. 329 are two cases of recent tuberculosis; they are living, and the knowledge that they are tuberculous is based entirely on a tuberculin test.

Cow No. 372 was found on autopsy to have a tuberculous affection limited to one post-pharyngeal lymph gland.

Cow No. 373 was found on autopsy to be affected with tuberculosis of the lung and liver and the mediastinal, portal, and mesenteric lymph glands. The lesions were widely scattered, but not extensive.

Cow No. 476 is living, is in good condition, and was used until recently, and would be in use now, as a private family cow, if a tuberculin test had not shown that she is affected with tuberculosis.

## EXAMINATION OF FECES FOR TUBERCLE BACILLI.

### MICROSCOPIC EXAMINATIONS.

Small portions of feces, taken at random from the interior of masses freshly passed by the cattle, were smeared on thin squares of glass, which are generally known in connection with microscopic work as cover-glasses. Similar preparations were made with material gently scraped from the mucous surface, just inside the anal orifice, of the rectum. The cover-glasses were dried, stained, and examined microscopically. Those made with material from the following cattle were found to contain bacteria identical in appearance with tubercle bacilli: Nos. 1, 84, 113, 325, 373, 374, and 476.

The number of bacilli per cover-glass varied with different cattle, and on different days with the same animal, from 0 to 25, and were usually a little more numerous in the rectal scrapings than in the feces. In some cover-glasses made with shreds of mucus picked from the feces the bacilli were much more numerous and in some instances innumerable.

Cover-glass preparations similarly made with feces and rectal scrapings from nontuberculous cattle were found to be free from anything resembling tubercle bacilli.

With a simple microscopic examination it is difficult to distinguish between tubercle bacilli and some harmless micro-organisms known as acid-fast bacteria. At first it was believed that the bacilli in the preparations belonged in whole or in part to the latter group, but this view was not tenable after it was discovered that similar bacilli could not be found in material from nontuberculous cattle.

## INOCULATION TESTS WITH GUINEA PIGS.

To establish absolutely that the bacilli were virulent tubercle germs, a series of inoculation tests with small animals was undertaken.

Two lots of guinea pigs were inoculated from each of the following cattle: Nos. 1, 84, 113, 325, 373, 374, and 384, one lot with feces and the other with scrapings from the rectal mucosa. Cattle Nos. 329 and 372 were omitted from the inoculation experiments, as they had given negative results with microscopic examinations, and cow No. 476 was received too late to be included.

The guinea pigs were inoculated under the skin on the inside of the right thigh; each received an amount of material equivalent in weight to about one-eighth of a grain, and more than 25 per cent of them became affected with septicemia and died before a tuberculous affection had time to develop. The results obtained with the animals that lived long enough to contract tuberculosis are as follows:

*Cow No. 1.*—Guinea pigs inoculated April 28, 1906, with feces did not become tuberculous; those inoculated on the same day with scrapings from the rectal mucosa were found on post-mortem examination to be affected with tuberculosis. Guinea pigs inoculated June 19, 1906, with feces, as well as those inoculated with scrapings from the rectal mucosa, were found on post-mortem examination to be affected with tuberculosis. Guinea pigs inoculated June 27, 1906, with feces, as well as those with scrapings from the rectal mucosa, remained free from tuberculosis.

We have from this cow one positive and two negative results with feces and two positive and one negative with scrapings from the rectal mucosa. It should be borne in mind that the subcutaneous inoculation of guinea pigs with cattle feces can not be regarded as a

delicate test for the presence of tubercle bacilli, and this is to nearly the same extent true of material scraped from the rectal mucosa, which is composed largely of feces. Cattle feces are a complex substance relative to the number and different kinds of bacteria contained. Each inoculation is followed by a local inflammation and suppuration, and hence the tubercle bacilli that are present, if their number is few, have every chance to be overcome by more rapidly growing organisms. The pathological conditions produced by the latter may in themselves be sufficient to prevent the tubercle bacilli from establishing themselves. That the acid-fast bacteria observed in the feces of cow No. 1 are certainly infectious tubercle bacilli is demonstrated absolutely by the occurrence of tuberculosis among the guinea pigs. That the feces and the scrapings from the rectal mucosa inoculated into guinea pigs that remained free from tuberculosis did not contain infectious tubercle bacilli can not be asserted positively. It is quite likely that the bacilli were present but did not have a chance to get actually into the tissues, and this view is supported by the fact that acid-fast bacteria were shown to be present in the feces of the cow on each of the three days on which the inoculations were made.

*Cow No. 84.*—Guinea pigs inoculated May 2, 1906, with feces did not become tuberculous; those inoculated with scrapings from the rectal mucosa were found on post-mortem examination to be affected with tuberculosis. Guinea pigs inoculated on other days from this cow died affected with septicemia before tuberculosis had time to develop.

*Cow No. 113.*—Guinea pigs inoculated with either feces or scrapings from the rectal mucosa on April 27, 1906; June 19, 1906, and June 27, 1906, were all found on post-mortem examination to be affected with tuberculosis.

*Cows Nos. 325, 373, 374, and 384.*—Guinea pigs inoculated with feces and scrapings from the rectal mucosa either died from septicemia before tuberculosis had time to develop or were found to be free from lesions of tuberculosis on post-mortem examination.

It was shown in another experiment[a] that the acid-fast bacteria microscopically demonstrated in the feces of cows Nos. 374 and 384 were virulent tubercle bacilli. The feces in the experiment referred to were fed to four hogs with the result that three of them became affected with tuberculosis.

The reasons why the inoculation of guinea pigs with feces is not a delicate test for the presence of tubercle bacilli do not apply when the feces are swallowed. There is no inflammation or suppuration in the intestine caused by swallowing large numbers of bacteria of the kind

[a] See Bulletin 88, Bureau of Animal Industry.

ordinarily contained in the feces of cattle. Bacteria, excepting those that are causative agents of specific diseases, if they pass through the uninjured wall of the stomach or bowels, are rapidly destroyed and cause no marked objectionable condition and no determinable pathological lesions. That tubercle bacilli, on the other hand, may pass through the uninjured wall of the intestine, without causing local lesions, is claimed on the basis of experimental evidence.

### SUMMARY OF FECES EXAMINATIONS.

To sum up the results as far as we have gone, among seven natural cases of tuberculosis, we have five, or 71.43 per cent, that were found on microscopic examination to be passing tubercle bacilli in their feces, and the two cattle that failed to show bacilli were a cow with an affection, strictly "closed," limited to one post-pharyngeal gland, and a young steer in which the presence of tuberculosis would not have been suspected without a tuberculin test.

The feces of four of the five cattle were used in inoculation tests and were found to be infectious in two cases. To these a third must be added, as subsequent inoculation tests hereinafter described will show.

Three cows that were fed culture of tubercle bacilli showed the presence of the bacilli in their feces on microscopic examination, and that the feces were infectious is shown for one cow by guinea pig inoculations and for the other two by the hogs that were fed with their feces in an earlier experiment. With this evidence we can not doubt that the tubercle bacilli that are swallowed by cattle pass in part through the entire length of the digestive tract and out through the rectum without losing their infectiousness.

Animals do not expectorate. The infectious material that is expectorated by tuberculous persons has its counterpart in tuberculous animals, but it is swallowed and not thrown out as sputum. The absence of real cavity formation in the lungs of cattle, such as occurs in tuberculous human lungs, does not seem to militate against the number of bacilli that may pass into the environment of tuberculous cattle. It must not be supposed that the occurrence of the bacilli in the feces of the cattle affected with naturally acquired tuberculosis was due to intestinal disease, as tuberculosis of the intestinal mucosa of cattle is an extremely rare affection.

## TESTS OF SALIVA, NASAL DISCHARGE, URINE, AND MILK.

The saliva, nasal discharge, and urine of two cows—Nos. 1 and 113—and the milk of three cows—Nos. 84, 372, and 373—were tested for tubercle bacilli by microscopic examination and guinea-pig inoculation, and the milk further by ingestion experiments.

The microscopic examinations were all negative,[a] and the inoculations were likewise negative, with the exception of the saliva of cow No. 113, which invariably produced tuberculosis. The milk was fed to a large number of guinea pigs for long periods of time, and other guinea pigs were injected with it, intra-abdominally, from time to time, without causing disease of any kind.

The urine of the cows was drawn from their bladders through a catheter with antiseptic precautions and placed for thirty minutes in the tubes of a small electrical centrifugal machine that made 2,000 revolutions per minute. As the specific gravity of tubercle bacilli is comparatively high, had any been present in the urine this treatement would have thrown them to the bottom of the tubes. Only the lower third of the urine in each tube was used for making intra-abdominal injections, and each guinea pig injected received a dose of 5 cubic centimeters.

The microscopic and inoculation tests with saliva, nasal discharge, and urine from cows Nos. 1 and 113 were twice repeated, giving three tests of each kind of material, each test on a different day. The milk from cows Nos. 84, 372, and 373 was removed from their udders with proper care to prevent its contamination with feces.

The absence of tubercle bacilli from the urine of cows Nos. 1 and 113, especially from that of the latter, indicates that they are rarely or never passed in the urine unless the genito-urinary organs are affected. The condition of the two cows relative to that of the other cattle strengthens this inference, as a larger number of positive results were obtained with the inoculation of their feces and scrapings from their rectal mucosa than with similar material from other cattle; and, further, the saliva of one of them (No. 113) infected every guinea pig inoculated with it. But neither the urine tests nor the cattle were sufficient in number for fully reliable negative conclusions, and this subject will receive further attention in the future if material for additional investigations can be obtained.

It was a source of surprise that no positive results followed the inoculations with nasal discharge, because the location of the nasal chambers seems well adapted for their frequent infection with material expelled from the lung. Cow No. 113 suffers with frequent severe paroxysms of coughing, during which her mouth is open and the air is largely, if not wholly, impelled through it. This may account for the presence of tubercle bacilli in her saliva and their absence from her nasal discharge.

[a] Subsequent microscopic examinations of the saliva of cow No. 113, in cover-glasses made from the sediment in tubes in which it was mixed with normal salt solution and then centrifugalized, showed the presence of tubercle bacilli.

Cow No. 1 is much less severely affected than No. 113, and the absence of positive results from inoculations with her saliva and nasal discharge is attributed to the infrequency and mildness with which she coughs. Her cough is rarely more than a gentle effort to clear her throat, and any substance expelled from her lung would barely pass her fauces and would be swallowed without reaching the forward portions of her mouth. The regurgitation and remastication of food that is practiced naturally by ruminants would tend to clear her throat as much as to infect it with swallowed infectious material. The infectious material expelled from the lung and swallowed does not necessarily lodge in the rumen, from which regurgitation takes place, and certainly does not become as thoroughly and evenly mixed with the contents of the rumen as it is with the feces.

The absence of positive results from the inoculations and feeding tests with milk is in perfect harmony with the writers' past experiences with milk from tuberculous cows with healthy udders when the milking is done with proper care against the introduction of infectious material that is not associated with the interior of the udder or the milk-secreting structures. The danger that milk may become infected from the environment of tuberculous cows is so great that we are justified in asserting that the few positive results we have had during many years with intra-abdominal injections of guinea pigs with milk from tuberculous cows with unaffected udders were not due to tubercle bacilli that were drawn with the milk, but to tubercle bacilli that dropped into it or into the milk pail from the exterior of the cow, despite all precautions to the contrary. Since it does not seem reasonable for tubercle bacilli to leave the body with the urine unless the genito-urinary apparatus is affected, similarly it does not seem reasonable for them to pass out with the milk through a healthy udder.

## FECES IN MILK THE REAL DANGER FROM TUBERCULOUS CATTLE.

The results of the tests point to the conclusion that the real danger from tuberculous cattle lies in the manner in which tubercle bacilli are disseminated with their feces.

The average number of acid-fast bacilli found in the feces of tuberculous cattle—leaving the greater number in the rectal scrapings and selected shreds of mucus out of consideration—is 6 per cover-glass. That these bacteria are tubercle bacilli has been sufficiently demonstrated by the positive results obtained with the inoculation tests and by their absence from the cover-glasses prepared with the feces of nontuberculous cattle. Now, let us calculate what this signifies as to the amount of infection that may be scattered by a single tuberculous cow in one day.

The cover-glasses were carefully weighed on a delicate balance before and after spreading a layer of feces on them. The amount of moist feces per cover-glass was found to have a maximum weight of one-thirtieth of a grain; it was usually less, and frequently not more than one-half as much. When one of the prepared cover-glasses is examined with the microscope the first condition noticed is that only a portion of it, rarely one-half, is sufficiently transparent for the detection of tubercle bacilli. It is reasonable to assume that the thicker, opaque portions of the film of feces are heavier and contain a larger number of bacilli than the lighter, transparent portions; hence, the tubercle bacilli that come into view after prolonged search are less than half the number actually present. To one who is familiar with microscopic work it would not appear unreasonable if we made the flat assertion that it is impossible to find more than 10 per cent of the tubercle germs that are present in cover-glasses of the kind we made for our examinations. But it is not necessary to present the fact in this extreme. If we take only the actual average number of tubercle bacilli seen, and the maximum weight of feces per cover-glass, we have 6 bacilli in one-thirtieth of a grain of feces, and we can not avoid feeling amazed at the amount of infection which this represents as leaving the body of a tuberculous cow daily.

A cow of average size passes about 30 pounds of moist feces each day, and if the whole of this mass could be spread on cover-glasses similar to our preparation, it would be sufficient to make 6,300,000 preparations, which would contain 37,800,000 microscopically demonstrable tubercle bacilli.

It is a significant fact that our microscopic examinations indicate that the bacilli in the feces, excepting those in the shreds and masses of mucus that do not enter into the present calculation, are evenly distributed, so that we do not have some portions of the feces that are very infectious and others that are innocuous. Practically it is all infectious, and every part of it is dangerous, and the mucus shreds alone show extreme infectiousness. The even distribution of the bacilli likewise indicates that they entered the intestine at the upper end of the digestive tract, as a considerable amount of churning, such as the food receives in the stomach and intestines, is required to effect this distribution.

Tuberculous persons from whom tubercle bacilli are being disseminated can be taught to use various precautions that will reduce to a minimum the danger to health in their environment. Sputa can be expectorated into receptacles containing germicidal fluids, and a cloth can be held before the mouth during paroxysms of coughing and at once treated so as to destroy the infectious material that is

impelled against or into it. Such persons can have individual sleeping apartments, and they should be prevented from coming into contact with articles of diet that are used by healthy persons. Their own individual generosity, when they are informed of their condition and its danger to health, will with rare exceptions induce them to take many precautions for the safety of their families and associates. Healthy persons, when they are informed of the danger involved in close contact with those affected with tuberculosis, and know that a chronic cough must be regarded as a very suspicious circumstance, can do much for their own protection by avoiding association on terms of close intimacy with persons who are actually or probably tuberculous.

With cattle we have a different state of affairs. Effective germicidal substances are too expensive and their proper application to large masses of feces daily is too difficult and troublesome for practical purposes. Feces are dropped everywhere in the environment of cattle—in stables, fields, and barnyards, and on roadways. They are splashed on the bodies of cattle, and frequently cattle lie down and get their bodies coated with them, and this is especially true of the parts of the body close to the udder. Feces are thrown against partitions of stalls and walls of stables and are promiscuously switched about by the soiled tails of animals, and no precaution or measure of cleanliness can entirely prevent them from getting into the milk pail occasionally and on the hands and clothing of the stable attendants and milkers frequently. We have seen large quantities of milk strained in many dairies, but have not found the dairy in which the milk was removed from the cows with a degree of cleanliness so perfect that the cloth or screen through which it was strained did not show the presence of some cattle hairs and fragments of a substance suspiciously like feces. In some dairies the quantity of feces that enters the milk pail, both fresh and dry, to judge from that which collects in the strainer through which it is poured, is comparatively large. The precautionary measures that can be used by the dairyman are limited by the price of milk. He can not afford to use measures the cost of which is so great that their application would convert his business into a philanthropic enterprise.

Regarding the dairy industry, we know the two following important facts: (1) That the commonest disease with which cows are affected is tuberculosis, and (2) that milk in some form reaches practically all persons. The person who does not use milk, cream, butter, or cheese is a rare exception to a general rule, and the family that uses no fresh dairy product can hardly be said to exist.

When we know how completely cattle feces may be charged with tubercle bacilli and how easily milk may be infected from this

source, and contemplate this fact, keeping in mind the wide distribution that dairy products have, and add to our knowledge some of the results recently obtained and published by competent investigators, we must conclude that the eradication of tuberculosis among cattle can not be too vigorously urged or pursued.

Our own investigations have shown that pulmonary tuberculosis is the most common form of the disease in animals, irrespective of the point at which the tuberculous infection enters the body, and that tubercle bacilli may pass through the intestinal wall and reach the lung without causing visible disease of the intestinal mucosa.

Nicholas and Descos and Ravenel proved by feeding healthy dogs on tuberculous fluids and examining the chyle in the thoracic duct a few hours later that tubercle bacilli may readily pass through the intestinal wall and infect the animal without causing lesions in the intestines.

For the benefit of readers who are not informed on the subject of anatomy it may be well to say that the thoracic duct is the common trunk of all the lymphatic vessels that drain the abdominal cavity and the organs contained in it, and an additional large portion of the body, and that it discharges its contents into the anterior vena cava, one of the large veins in which the blood is returned to the heart. The material discharged by the duct into the vein is carried with the blood directly to the heart and pumped by the heart to the lung, where it is filtered through the exceedingly fine capillary network in which the blood is arterialized. It can readily be seen that tubercle bacilli that have passed through the intestinal wall and through the lymphatics into the thoracic duct have a clear way to reach and infect the lung.

Vallée calls attention to the fact that the lung is the favorable location of the tubercle bacillus in all species of animals, and he made experiments that indicate that infection through the digestive tract constitutes a mode of inoculation which is extremely favorable to the production of pulmonary tuberculosis. He concludes that ingestion is the quickest and most certain method for the tubercularization of the lymph glands associated with the lung, and that the tubercle bacillus may pass through the intestinal wall without producing appreciable lesions in the mucous membrane of the intestine or in the mesenteric lymph glands.

Schlossman and St. Engle found that tubercle bacilli introduced into the stomachs of guinea pigs by means of a laparotomy could be found in the lungs in a few hours. Calmette produced pulmonary tuberculosis of a goat by introducing suspensions of tubercle bacilli into its rumen. Cadéac asserts after careful investigation that the

widely accepted hypothesis of the transmission of tuberculosis by inhalation of dust from dried sputa has not been proven; and he showed that sputum dries slowly, is difficult to pulverize, and rapidly loses its infectious character.

### PERSONS PROBABLY CONTRACT TUBERCULOSIS CHIEFLY BY INGESTION.

It has long been supposed that the frequency with which tuberculosis occurs among persons is due to the direct infection of the lung (probably because the lung is most frequently affected) with sputum which has become dried and pulverized after being expectorated by persons affected with tuberculosis. This supposition or theory is gradually being shown to be erroneous, (1) because sputum does not pulverize easily and loses its infectious character in a short time; (2) because the greater frequency with which the lung is affected as compared with any other organ of the body is accounted for in a way that does not require the acceptance of the theory of direct infection from the air, and (3) because it is being shown by a number of investigators that tubercle bacilli may pass through the intestinal wall without injuring it and thus may reach the lung, and that the ingestion of tubercle bacilli is the quickest and simplest way to infect the lung. In other words, the inhalation of tubercle bacilli is losing much of its importance in the minds of investigators, and the swallowing of tubercle bacilli is gradually supplanting it as the true mode of infection. It therefore becomes necessary to give the most careful attention to the sources from which tubercle bacilli may reach our daily food and drink.

There is little doubt that many persons become affected with tuberculosis by breathing tubercle bacilli in an infected environment. Infectious material sprayed from the mouths of tuberculous persons lodges in the upper air passages, is drawn or drops into the mouth, and is swallowed and taken up by the intestines, and through the lymph channels reaches the lung. Other cases result from tuberculous persons who are allowed to handle and prepare food and to clean and care for kitchen and table utensils.

But there is little doubt in the writers' minds that tubercle bacilli from no source receive a wider distribution in a perfectly fresh state than those that enter the milk with fragments of feces from tuberculous cows. As we have already pointed out, tuberculosis is the most frequent disease with which dairy herds are affected, and milk has an enormously wide distribution. It is taken everywhere on every day and reaches practically every person on every day.

INFECTIOUSNESS OF SOILED MILK SHOWN BY INOCULATION OF GUINEA PIGS.

These facts make it desirable to know something definite about the significance of the frequently unavoidable introduction of feces into milk, and we have tried to gain light on the subject by inoculating guinea pigs with milk into which small fragments of feces from tuberculous cows were placed in a manner as nearly as possible like that which occurs in a dairy stable.

Normal, fresh milk from healthy cows was soiled with small masses of feces from cows Nos. 1, 84, 113, 325, and 373. The amount introduced into each sample of milk was very small, about as much as would enter in proportion to the volume of milk in a dairy stable in which average cleanliness is practiced. Some of the soiled milk was injected without further treatment and some was strained through linen cloth and then injected. The injections were intra-abdominal, and each guinea pig received a dose of 5 cubic centimeters.

To make sure that the milk used in this experiment was free from infectious material before it was soiled with the feces, a number of guinea pigs were injected with it from time to time in its pure unsoiled state. Such guinea pigs remained well until they were killed, and on post-mortem examination were found to be free from lesions of disease.

The following tables give the results obtained with the injections of soiled milk. No table is given for cow No. 325, as no tuberculosis was caused by the milk soiled with her feces. In all other respects the inoculations were identical with those of cow No. 373.

*Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 1 (tuberculous).*

MILK NOT STRAINED.

| No. of guinea pig. | Date of injection. | Date of death.[a] | Remarks. |
|---|---|---|---|
| 7608 | June 19, 1906 | July 24, 1906 | Tuberculosis of liver, spleen, and abdominal lymph glands. |
| 7609 | .....do..... | .....do..... | No lesions of disease. |
| 7610 | .....do..... | .....do..... | Tuberculosis of mesenteric glands. |
| 7611 | .....do..... | .....do..... | Tuberculosis of liver, spleen, and abdominal lymph glands. |

MILK STRAINED THROUGH CLOTH.

| | | | |
|---|---|---|---|
| 7614 | June 19, 1906 | July 21, 1906 | Generalized tuberculosis. |
| 7615 | .....do..... | .....do..... | Do. |
| 7616 | .....do..... | June 25, 1906 | Died, affected with peritonitis. |
| 7617 | .....do..... | June 21, 1906 | Do. |

[a] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

*Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 113 (tuberculous).*[a]

MILK NOT STRAINED.

| No. of guinea pig. | Date of injection. | Date of death.[b] | Remarks. |
|---|---|---|---|
| 7503 | May 29, 1906 | July 23, 1906 | Generalized tuberculosis. |
| 7504 | do | do | Tuberculosis of liver and spleen. |
| 7505 | do | do | Do. |
| 7506 | do | do | Generalized tuberculosis. |
| 7507 | do | do | Tuberculosis of liver, spleen, and abdominal lymph glands. |
| 7508 | do | do | Do. |
| 7573 | June 9, 1906 | do | Generalized tuberculosis. |
| 7574 | do | do | Do. |
| 7575 | do | do | Do. |
| 7576 | do | do | Do. |
| 7592 | June 18, 1906 | July 24, 1906 | Do. |
| 7593 | do | do | No lesions of disease. |
| 7594 | do | do | Tuberculosis of liver. |
| 7595 | do | do | Tuberculosis of spleen. |

MILK STRAINED THROUGH CLOTH.

| | | | |
|---|---|---|---|
| 7596 | June 18, 1906 | July 23, 1906 | Tuberculosis of liver and spleen. |
| 7597 | do | do | Generalized tuberculosis. |
| 7598 | do | do | Tuberculosis of liver, spleen, and abdominal lymph glands. |
| 7599 | do | do | Do. |

[a] In connection with this cow it should be observed that 17 of a total of 18 guinea pigs inoculated with milk that was soiled with her feces became affected with tuberculosis, and that the amount of feces added to the milk was so small that not one guinea pig of the 18 that received intraperitoneal injections became affected with peritonitis.

[b] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

*Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 84 (tuberculous).*

MILK NOT STRAINED.

| No. of guinea pig. | Date of injection. | Date of death.[a] | Remarks. |
|---|---|---|---|
| 7638 | June 21, 1906 | July 25, 1906 | Tuberculosis of lung, liver, and spleen. |
| 7639 | do | July 5, 1906 | Died, affected with peritonitis. |
| 7640 | do | July 25, 1906 | Tuberculosis of liver and spleen. |
| 7641 | do | June 26, 1906 | Died, affected with peritonitis. |
| 7642 | do | July 25, 1906 | Tuberculosis of liver and spleen. |

MILK STRAINED THROUGH CLOTH.

| | | | |
|---|---|---|---|
| 7643 | June 21, 1906 | July 25, 1906 | Tuberculosis of liver and spleen. |
| 7644 | do | June 22, 1906 | Died, affected with peritonitis. |
| 7645 | do | do | Do. |
| 7646 | do | do | Do. |
| 7647 | do | do | Do. |
| 7673 | June 22, 1906 | July 24, 1906 | No lesions of disease. |
| 7674 | do | do | Do. |
| 7675 | do | do | Do. |
| 7676 | do | do | Do. |
| 7677 | do | do | Do |
| 7678 | do | do | Do. |

[a] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

*Results of injecting guinea pigs with normal milk (from healthy cows) soiled with feces from cow No. 373 (tuberculous).*

MILK NOT STRAINED.

| No. of guinea pig. | Date of injection. | Date of death.[a] | Remarks. |
|---|---|---|---|
| 7624 | June 20, 1906 | July 24, 1906 | No lesions of disease. |
| 7625 | .....do..... | .....do..... | Do. |
| 7626 | .....do..... | .....do..... | Do. |
| 7627 | .....do..... | .....do..... | Do. |
| 7549 | June 7, 1906 | June 9, 1906 | Died, affected with peritonitis. |
| 7550 | .....do..... | June 16, 1906 | Do. |
| 7551 | .....do..... | July 23, 1906 | Generalized tuberculosis. |
| 7552 | .....do..... | .....do..... | Do. |
| 7553 | .....do..... | .....do..... | Do. |
| 7554 | .....do..... | .....do..... | Do. |

MILK STRAINED THROUGH CLOTH.

| | | | |
|---|---|---|---|
| 7630 | June 20, 1906 | July 24, 1906 | No lesions of disease. |
| 7631 | .....do..... | .....do..... | Do. |
| 7632 | .....do..... | .....do..... | Do. |
| 7633 | .....do..... | .....do..... | Do. |

[a] Except where the word "died" is used in the remarks, the guinea pigs remained alive until they were killed for post-mortem examination.

As the tables show, both the strained and unstrained milk soiled with feces from cows Nos. 1, 84, and 113, and the unstrained milk soiled with feces from cow No. 373, were infectious for guinea pigs. Add to this cow No. 325 with negative results, and we have one cow that was fed tubercle bacilli in her drinking water and four affected with naturally acquired tuberculosis. The former and three of the latter were passing tubercle bacilli in their feces in sufficient numbers and of sufficient virulence to make the infection of milk exposed in their environment easily possible. No better evidence can be presented to support the conclusion that the presence of a single tuberculous cow in a dairy stable, even if her milk is not used, should not be tolerated. She is a direct danger to the healthy cattle that are exposed to her, and may be responsible for the introduction of infectious material into their milk, and thus make it dangerous for use by persons or animals.

It must not be supposed that the cows used in these tests were old, invalid animals, so badly affected with tuberculosis that no conscientious dairyman would retain them in his herd. With the single exception of cow No. 113, their condition, as far as could be determined by a simple, unaided, physical examination, was better than that of the majority of dairy cows in actual use. They looked better nourished, and their hair was smoother, glossier, and cleaner. The slight, infrequent cough with which they were affected would not have attracted the attention of the casual observer, and might have been honestly attributed by most dairymen to dust in the air of the

stable. These cows illustrate anew the fact that the danger from tuberculosis does not rest on an intensely infectious character of the disease, but on its insidiousness and concealed and secret nature, which enables it to come and develop and spread infection without warning. Strictly speaking, the best as well as the worst cow in appearance relative to health must be suspected until she has been proven sound by a tuberculin test.

## ADDITIONAL FEEDING AND INOCULATION TESTS.

Two additional tests were made for the presence of tubercle bacilli in the environment of tuberculous cattle. (1) The mixed feces of cows Nos. 1 and 113 were fed, together with cornmeal and bran, to four healthy hogs, and (2) earth from the outside of the stable in which the tuberculous cattle were housed, near the door through which the manure was removed, was microscopically examined and used for guinea-pig inoculations.

Before the feeding experiments began the hogs used in them were tested with tuberculin without showing a reaction, and hence must be regarded as originally free from tuberculosis. The feeding was continued about six weeks, and shortly after it was stopped the hogs were again tested with tuberculin. With the second tuberculin test all four gave the reaction indicative of the presence of tuberculosis. When the hogs were killed and examined post-mortem it was found that one was affected with tuberculosis of the lung and liver and the submaxillary, prescapular, and mesenteric lymph glands, and the other three with tuberculosis of the submaxillary lymph glands. The submaxillary glands, not alone from the results obtained with this experiment, but also from other observations on a larger number of animals, must be regarded as the first structures in the bodies of hogs to become affected when they contract tuberculosis through the ingestion of infectious material.

The fact that the four hogs contracted tuberculosis shows that the feces contained active, virulent tubercle bacilli, and likewise, as was pointed out in a former publication,[a] that much of the tuberculosis that occurs among hogs may be due to a method of feeding extensively practiced in the West—turning hogs behind cattle—when insufficient care is taken against the presence of tuberculosis among the cattle. Hogs that are exposed to cattle feces or have access to manure heaps from stables in which cattle are kept may at any time become tuberculous if the cattle are affected. This practice and the feeding of skim milk, especially when tubercle bacilli have been concentrated in it by the separation of the cream from the milk with a centrifugal

[a] Bulletin 88, Bureau of Animal Industry.

machine, are probably the most frequent causes of tuberculosis among hogs. That tubercle bacilli may be dropped, splashed, sprayed, or switched into milk when it is obtained from tuberculous cows or has been exposed to the environment of tuberculous cattle has already been pointed out, but can not be too often repeated.

The microscopic examination of the earth from the outside of the stable in which the tuberculous cattle used in these experiments were kept revealed the presence of numerous acid-fast bacilli. Most of the guinea pigs inoculated with specimens of this earth died from septicemia, and those that lived until they were killed for post-mortem examination showed either no lesions of disease (in a few instances) or very extensive lesions that could easily be mistaken for tuberculosis, but were shown by microscopic examination to be nontuberculous and due to the action of other bacteria than tubercle bacilli. Hence we can not assert definitely that the acid-fast bacteria found in the samples of earth on microscopic examination were tubercle bacilli, as acid-fast bacteria are very common in the soil in and around stables and barns.

## CONCLUSIONS.

1. Tubercle bacilli are disseminated with the feces of tuberculous cattle. This is shown to be the case by microscopic examination, by inoculation tests with guinea pigs, and by ingestion experiments with hogs.

2. Feces are the most dangerous factor in the dissemination of tubercle bacilli by cattle affected with tuberculosis. In this respect feces must be regarded as having a place with cattle similar to that commonly accorded to sputa with tuberculous persons.

3. It is not alone the feces of visibly affected cattle which disseminate tubercle bacilli in a way that is dangerous to man and animals, but also the feces of cattle so slightly affected that the diagnosis of tuberculosis with them depends entirely on the application of the tuberculin test.

4. Tubercle bacilli that are swallowed by cattle are to a great extent passed entirely through the digestive tract and out with the feces without loss of infectiousness. As cattle do not expectorate, the infectious matter that is coughed up from their lungs is swallowed, passed through their bodies, and scattered with their feces.

5. Bacilli may reach the environment of tuberculous cattle from their mouths, but this is evidently of rare occurrence compared with the dissemination through feces, especially when the cattle are not visibly tuberculous.

6. The nasal discharge of tuberculous cattle was found to be free from infectious material. Cow No. 113 is so severely affected with

tuberculosis that this freedom in her case seems to indicate that nasal discharge rarely contains tubercle bacilli. It is possible that a larger number of tests may modify this conclusion.

7. Urine is probably free from tubercle bacilli when the genito-urinary organs are not affected and no infectious material has been introduced into it after it has been passed. The practical significance of this conclusion lies in its bearing on the question whether tubercle bacilli are ever thrown out by tuberculous subjects through unaffected secretory organs with otherwise normal secretions.

8. Milk from tuberculous cows with unaffected udders we believe to be free from infection until it has become contaminated with feces or some other material that contains tubercle bacilli from the outside of the cows or from their environment; that is to say, it is not believed that tubercle bacilli are eliminated with the milk from tuberculous cows unless disease of the udder or structures connected with it is present. This conclusion is drawn from the present series of investigations and is supported by our earlier work relative to the milk of tuberculous cows. The present investigations include only a few cows and a comparatively small number of guinea pigs. The earlier investigations extend over a dozen years, during which milk from scores of tuberculous cows was injected into the abdominal cavities of hundreds of guinea pigs.

When milk injections into guinea pigs are made by pathologists or bacteriologists as a test for the presence of infectious material, unusual, though not always sufficient, precautions are taken at the time of milking to protect the milk from contamination with foreign matter of any and every kind that may reach it from the exterior of the cow or her environment. It follows that the scientific injections give nearly accurate results as to the frequency with which tuberculous cows pass tubercle bacilli with their milk; but they give no data at all as to the frequency with which milk from tuberculous cows, or healthy cows in a tuberculous environment, contains infectious material when it is drawn and handled with the ordinary precautions that a dairyman can economically practice.

The observations made by the writers definitely show that the frequency with which milk contains tubercle bacilli is greatly underestimated, especially when it is milked in the customary way from tuberculous cows with healthy udders, or from entirely healthy cows in a tuberculous environment.

9. It has been positively shown that the introduction of a small quantity of feces from tuberculous cattle into normal milk is equivalent to the introduction of a sufficient amount of infectious material to cause a generalized tuberculosis in guinea pigs that are given intra-abdominal injections of small amounts of such soiled milk. This

is true not only with the feces of a severely affected cow, like No. 113, or a cow that is swallowing cultures of tubercle bacilli, like No. 84, but also with the feces of cows that are not known to be affected with tuberculosis until a tuberculin test or post-mortem examination has been made, as cows Nos. 1 and 373.

The quantity of feces introduced into the milk was no greater than frequently enters with ordinary milking.

10. We are unacquainted with any means by which it can be determined when cattle or their feces become dangerous to the health of persons or animals; hence every cow known to be affected with tuberculosis must be regarded as positively dangerous. Physical condition gives no information from which it is possible to determine how seriously a cow is affected with tuberculosis or how freely she is scattering tubercle bacilli. Cattle affected with advanced tuberculosis from which infection is being disseminated in a dangerous way may retain the appearance and give the general impression of perfect health. Frequently nothing abnormal can be detected about them after the most searching examination by the owner or even by a trained veterinarian; and besides it is not customary to make careful examinations or to employ professional men to do so until cattle show marked symptoms of disease.

11. In order to guard against the spread of tuberculosis among cattle and other animals, and more especially for the protection of persons, every dairy cow should be periodically tested with tuberculin, and every cow that shows a reaction indicating that she is affected with tuberculosis should at once, regardless of her general appearance or condition or semblance of health, be removed from use as a dairy cow and from all contact with dairy cattle or other healthy animals. If segregation is practiced, it should be complete, so that no healthy animal will be exposed to feces that may swarm with living, virulent tubercle bacilli.

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